Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming

Posch, Thomas ; Köster, Oliver ; Salcher, Michaela M ; Pernthaler, Jakob

Abstract: Anthropogenic-induced changes in nutrient ratios have increased the susceptibility of large temperate lakes to several effects of rising air temperatures and the resulting heating of water bodies1. First, warming leads to stronger thermal stratification, thus impeding natural complete water turnover (holomixis), which compensates for oxygen deficits in the deep zones, Second, increased water temperatures and nutrient concentrations can directly favour the growth of harmful algae. Thus, lake-restoration programmes have focused on reducing nutrients to limit toxic algal blooms. Here we present evidence that the ubiquitous harmful cyanobacterium Planktothrix rubescens has become the dominant species in a large lake during the past four decades, although the phosphorus content of the ecosystem decreased fivefold. However, the nitrogen input was not diminished concomitantly, favouring this non-N2-fixing cyanobacterium owing to increased N:P ratios. P. rubescens contains gas vesicles that allow for buoyancy to accumulate within the depth of optimal irradiance. As the toxic cyanobacterium has low consumption by predators, water turnover represents the main mechanism of seasonal population control. Thus, unidirectional lake-restoration measures in parallel with recurrent absence of holomixis owing to lake warming may lead to similar undesired effects that have formerly emerged from fertilization.

DOI: https://doi.org/10.1038/nclimate1581

Harmful filamentous cyanobacteria favoured

by reduced water turnover with lake warming

Thomas Posch[1*], Oliver Köster[2], Michaela M. Salcher[1] & Jakob Pernthaler[1]

[1] Limnological Station, Institute of Plant Biology, University of Zurich, Seestrasse 187, CH-8802 Kilchberg, Switzerland.

[2] Zurich Water Supply (WVZ), Hardhof 9, CH-8021 Zurich, Switzerland.

Number of pages (incl. references, correspondence, acknowledgements, author contributions and figure legends): 15; Figures: 4

Intended as Letter in Nature Climate change

*Corresponding author:
PD Dr. Thomas Posch
Limnological Station, Institute of Plant Biology, University of Zurich
Seestrasse 187, CH-8802 Kilchberg, Switzerland, Europe
Phone: 0041 44 716 1224
Fax: 0041 44 716 1225
e-mail: posch@limnol.uzh.ch

Anthropogenic induced changes in nutrient ratios have increased the susceptibility of large temperate lakes to several affects of rising air temperatures and the resulting heating of water bodies[1]. First, warming leads to stronger thermal stratification, thus impeding natural complete water turnover (holomixis) which compensates for oxygen deficits in the deep zones[2,3]. Second, increased water temperatures and nutrient concentrations can directly favour the growth of harmful algae[4,5,6]. Thus, lake restoration programs have focused on reducing nutrients to limit toxic algal blooms [7]. Here we present evidence that the ubiquitous[8,9,10] harmful cyanobacterium *Planktothrix rubescens* has become the dominant species in a large lake during the last 4 decades, although the phosphorus (P) content of the ecosystem decreased by fivefold. However, the nitrogen (N) input was not diminished concomitantly, favouring this non N_2-fixing cyanobacterium due to elevated N:P ratios[10]. *Planktothrix rubescens* contains gas vesicles that allow for buoyancy to accumulate within the depth of optimal irradiance[11]. Since the toxic cyanobacterium has low consumption by predators[12], water turnover represents the major mechanism of seasonal population control. Thus, unidirectional lake restoration measures[13] in parallel with recurrent absence of holomixis due to lake warming may lead to similar undesired effects that have formerly emerged from fertilization.

Nutrient ratios of numerous temperate lakes have drastically changed due to discharge of untreated sewage[7,13]. The consequences of fertilization (eutrophication) included the development of algal blooms[7] and decreasing oxygen (O_2) concentrations in deep water layers due to the aerobic decomposition of sinking plankton biomass[14]. Whereas eutrophication in industrialized countries is mainly manageable by the reduction of nutrient inputs, other effects of anthropogenically induced global change on lakes are definitely out of control at present: Increasing air temperatures cause a heating of the surface water masses (epilimnion) of lakes. This directly affects the physiology of planktonic organisms, leading to taxonomic shifts[15,16]

and potentially to a loss of biodiversity[17]. Warming also leads to stronger thermal stratification of large lakes due to the temperature dependent density difference between the epilimnion and the deep water zones (hypolimnion). Depending on geographical position and morphometry, water turnover (mixis) of temperate lakes occurs once or twice a year, when density gradients between epi- and hypolimnion reach a minimum during cold seasons. Mixis is lake-type specific and at optimal conditions reaches the deepest zone. The frequency and intensity of water turnover is critical for lakes, as it is the only time when hypolimnetic O_2 deficits can be compensated. Thus, increased thermal stability may impede holomixis and can negatively affect the O_2 budget[1,2].

This raises the question of what cumulative effects can appear when lake warming works in parallel with altered nutrient ratios. Here we present a 40 year data set, documenting that such cumulative effects favour the mass occurrence of a ubiquitous toxic cyanobacterium (*P. rubescens*) in a Swiss pre-alpine lake (Lake Zurich; a drinking water source for 1.5 million people). Our analysis suggests that it has the potential to develop into a dominant species in many large temperate lakes, as physiological characteristics of *P. rubescens* differ from other expansive cyanobacteria in several aspects[18,19]. We show that the success of this species was inversely related to the magnitude of phosphorus. Measurements of total phosphorus (TP) in Lake Zurich pointed to a dramatic increase at the mid of the 20th century until the early 1970s. Our data set -starting in 1972- documents the re-oligotrophication[20] period, i.e., an exponential decrease of the TP content from 360 to 75 metric tons (Fig.1a). In contrast to TP, nitrate-nitrogen (NO_3-N) linearly increased from 1972 until 1995 (linear regression, r^2=0.86, $p<0.0001$). As a consequence of re-oligotrophication, pronounced surface blooms of eukaryotic phytoplankton species were no longer observed. Although the TP content decreased by a factor of 4.8 during the last 40 years, annual means of total phytoplankton fresh weight (=biomass) did not decline (Supplementary Table S1), with an average of 3,500 ± 870 metric tons (Fig.1b). The apparent stability of the autotrophic biomass

is based on two opposing trends. While the eukaryotic algal biomass steadily declined to 1,670 metric tons, the annual average biomass of a single cyanobacterial species (*P. rubescens;* Fig.1b, Supplementary Fig.S1 and Table S1) continuously increased. Since the 1990s, *P. rubescens* represents on average 40% of the total annual autotrophic biomass, while it only represented 11% in the two previous decades. Notably, the appearance of the cyanobacterium is a lake-wide phenomenon[21]. During the strongest eutrophication of the lake (early 1970s), the N:P ratio (Fig.1c) of nutrients was close to the Redfield ratio (16:1). At that time eukaryotic phytoplankton clearly dominated the ecosystem (Fig.1c). However, with the exponential decline of dissolved P (DP) and still high N concentrations, the N:P ratio strongly increased during the last 40 years concomitantly with an increase of the cyanobacterium (Fig.1c, Supplementary Table S1). This observation is in contradiction to predictions about the expansion of other cyanobacterial species, but is reasonable if considering that *P. rubescens* is unable to fix N_2 directly but depends on inorganic N sources[10] (mainly NO_3). It should be stressed that there are neither a direct statistical correlation between dissolved N and *P. rubescens* nor a synchronous chronological trend (insert in Fig.1c, Supplementary Table S1). Thus, dissolved nitrogen concentrations seem to be far in excess of the actual demands by organisms.

P. rubescens is considered toxic for most eukaryotic organisms (including humans)[22] due to cyclic heptapeptides (microcystins) which are potent hepatotoxins and also act as efficient inhibitors of highly conserved protein phosphatases[23]. Potential consumers (zooplankton) come into contact with the endotoxins when cyanobacteria are digested[12]. *P. rubescens* shows several other physiological traits which differ from those of other bloom forming phytoplankton including extremely slow growth rates[24], low temperature optima for growth[18], survival in the cold and dark for weeks[25], photoheterotrophy[26], and an adaptation to low-light[19,24]. Thus, it grows preferably below the surface in the zone with the steepest temperature gradient (metalimnion)[11,24]. Filaments consist of several hundred cells (5x5µm),

reaching a length up to 3,000µm (Supplementary Fig.S1). Cells contain gas vesicles allowing for buoyancy and for a vertical migration velocity of 0.4-0.8m day^{-1}. The most dominant of the three gas vesicle genotypes in Lake Zurich (GV3) possesses the strongest vesicles which can withstand a critical collapse pressure of 1.17MPa, i.e. they remain buoyant down to a depth of 99m during mixis[27].

The annual growth cycle of *P. rubescens* starts during the period of stable thermal summer stratification (Fig.2a, Supplementary Fig.S2), reaching a maximum in the epilimnion during autumn. With the loss of thermal stratification, filaments become increasingly entrained in deeper waters. Usually holomixis of Lake Zurich is expected in March/April, causing a transport of *P. rubescens* into the hypolimnion (Fig.2a). The minimum of cyanobacterial biomass is observed during early summer (May to July).

The strength of water mixis seems to have a pronounced affect upon the success of *P. rubescens* in the subsequent months; e.g. in March 1999, holomixis could be deduced from the homogenous vertical distribution of what had initially been the concentrated cyanobacterial population and from equal O_2 concentrations throughout the water column (Fig.2b). In June 1999, the cyanobacterium was below the limit of detection (<0.2 filaments ml^{-1}). The physical entrainment of filaments into the deep hypolimnion is the most significant mortality factor for *P. rubescens*. Firstly, gas vesicles within cells collapse in depths below 100m due to hydrostatic pressure[27]. Secondly, although some filaments below 80m will remain buoyant[28], their ascending velocity is too slow for reaching the metalimnetic population during early summer[25]. While the lake underwent holomixis in 1999, the mixing zone in March 2001was reduced to the upper 80m (Fig.2b). No oxygen enrichment of the deep water masses was observed. In the following summer, a pronounced cyanobacterial bloom developed in the metalimnion. In our analysis of the long-term data-set, we determined the depth layers above which 90% of the total population was situated (the 90% isocline;

Fig.2c). A significant chronological trend of decreasing depths of the 90% isoclines (Supplementary Fig.S3d and Table S1) indicated a declining mixis intensity.

Warming impedes holomixis of Lake Zurich

Since 1988, there were positive deviations of annual air temperatures (mean=0.57°C, max.=1.35°C; Fig.3a) from the 40-years average in all but 3 years. Annual surface water temperatures closely mirror the temporal trend of air temperatures (Supplementary Fig.S3a). Water temperatures from 1988 onwards were also higher than the average (mean deviation=0.42°C, max.=1.13°C) in all years (except for 1996). The probability of holomixis can be deduced from the minimal temperature difference between the epi- and the hypolimnion during the period of mixis (Fig.3b; Supplementary Fig.S3b). Values around zero indicate homogenous temperatures (homothermie) throughout the whole water column, which is a prerequisite for wind-induced water circulation. Negative differences were found in years with very cold winters, i.e. the epilimnion was up to -0.4°C cooler than the hypolimnion (around 4°C). Although such negative differences can temporarily impede water turnover, homothermie will eventually occur upon vernal warming allowing for holomixis. By contrast, positive temperature differences will permanently impede the mixis of the less dense surface water layers with the colder hypolimnion.

Due to bacterial respiration O_2 concentrations in the deepest zone of Lake Zurich are below the limit of detection in autumn and early winter. During holomixis this O_2 deficit can be compensated (Fig.2b). Until the 1990s, the hypolimnetic O_2 content of Lake Zurich was stable at 17,000 metric tons (Fig.3c). The first drastic decline in the total O_2 level (<9,000 metric tons) was documented from 1988 until 1990, a period characterized by three consecutive warm winters (Fig.3b). Subsequently, the frequency of partial mixis increased, and a drastic decline in hypolimnetic O_2 content was regularly observed (Fig.3c; Supplementary Fig.S3c and Table S1).

The first report of the occurrence of *P. rubescens* in Lake Zurich dates back to 1899, and it regularly appeared until 1964. During the strongest period of eutrophication, *P. rubescens* was below the limit of microscopic detection. A reestablishment of the cyanobacterium was observed in the late 1970s. After a decade characterized by negligible biomass (Fig.3d; Supplementary Fig.S2), the first in a series of weak mixis events coincided with the permanent establishment of *P. rubescens* in the lake. Taking an O_2-based proxy for water turnover intensity (Supplementary Figs S3c and S4), the mixis depth only reached 88m in 1989 (Fig.3d, Fig.4a) and 90% of the *P. rubescens* population were localized above 74m (Supplementary Fig.S3d). Since then, the scenario of extremely weak mixis with its favourable effects on cyanobacteria has increasingly emerged as the rule rather than an exception (Fig.3d; Supplementary Fig.S5 and Table S1). In the last decade water turnover no longer reached the deepest zones anymore, and the cyanobacterium typically persists throughout the whole year (Supplementary Fig.S2). We estimate that up to 100% of the spring biomass of *P. rubescens* could survive the hypolimnetic entrainment in years of partial mixis (Fig.4b). The importance of mixing on the annual population development is also reflected in the highest influence of the spring population (January to April) on subsequent growth phases, extending even to the population one year later (Fig. 4c). Additionally, the strength of mixis determines the amount of DP reintroduced into the epilimnion each spring (Fig.4d). Thus, the chronological decline of DP due to restoration measures is further enhanced by weak mixis events.

Warming and altered nutrient ratios

Here we document that a ubiquitous toxic cyanobacterial species is promoted by cumulative effects that appear when lake warming works in parallel with altered nutrient ratios (Supplementary Fig.S5). The lack of holomixis (Figs 4a & 4b) and elevated water temperatures (Fig.4e) directly favour the seasonal growth of *P. rubescens*, whereas the changed N:P ratio appears to be the prerequisite that the cyanobacterium could establish in

Lake Zurich at all. In the past, local restoration measures focused primarily on the reduction of P. While this strategy was successful in terms of reduction of eukaryotic phytoplankton blooms it obviously caused an ideal nutrient stoichiometry for this toxic cyanobacterial species. Our study highlights the paradoxical situation that the extensive but unidirectional restoration programs[13] for improving O_2 budgets and limiting harmful algal blooms may increasingly be counterbalanced by the effects of climate change.

Methods summary

Lake Zurich (area=66.2km^2, maximal depth=136m) is situated within a densely populated, economically important region in Switzerland[29]. It serves as recipient for 24 sewage treatment plants and is a major source of drinking water. Depth related volumetric data were calculated with the truncated cone equation based on isobath areas[28] (Supplementary Fig.S6b).

Monitoring program. Since 1972, the Zurich Water Supply (WVZ) conducts an intensive biological, physical and chemical monitoring at the location of the maximal water depth (136m). The following parameters were determined monthly for 19 depths (Supplementary Fig.S6b) during 40 years (n=9,120): total phosphorus, nitrate-nitrogen, dissolved oxygen, temperature[20]. Dissolved phosphorus and ammonium-nitrogen were determined at the same spatial and temporal resolution starting from the year 1976. Data on total phytoplankton and *Planktothrix rubescens* specific biomass (n=6,720) were determined monthly for 14 depths (Supplementary Fig.S6). The quantification of phytoplankton is based on microscopic evaluation of fixed samples[20].

Cumulative values. Based on standard sampling depths, we calculated values in 1m steps for the whole water column by linear interpolation (0-136m). These values were multiplied with the corresponding water volume of each depth and summed up to obtain the total amount (in metric tons) of oxygen, nutrients and biomasses (n=65,280 as 136 depth values * 12 months *

40 years). The precision of the estimates was confirmed using highly depth-resolved data (Supplementary Fig.S6a).

Temperature. Homogenized monthly air temperatures[30] were measured at the Zurich/Fluntern Station. Winter air temperatures are means of values from December to February. Deviations of air temperatures were calculated from the average during the whole investigation period (1972-2011). Mean annual water temperatures were based on volume weighted values.

Mixing depth. The seasonality of oxygen concentrations for the depths 120m-135m was analysed for the period 1972-1990 (Supplementary Fig.S4). The highest oxygen concentrations were reached in April (median = 6mg O_2 l^{-1}). This value was regarded as a proxy for mixis intensity.

Statistical analyses. All analyses were performed with the Microsoft EXCEL add-in programs XLSTAT 2012-TIME and -ADA. Trends in the long term data series (Supplementary Table S1) were tested by the Mann-Kendall trend test and the seasonal Mann-Kendall test (period = 12). Prior to analysis, data series were tested for autocorrelation. In cases of autocorrelations, Mann-Kendall trend tests were calculated using the Yue and Wang method. P-values of the statistical tests were related to a significance value of alpha = 0.05. The null hypothesis H0 states that there is no trend in the data series, while the alternative hypothesis HA states that there is a trend. In cases where p-values were smaller than alpha=0.05, the null hypothesis HO was rejected. Redundancy analysis (Supplementary Fig.S5) was used to determine the effects of environmental variables on algal and *P. rubescens* densities. Prior to statistical analyses, data were log(x+2)-transformed to obtain normal distribution. Only those variables exhibiting a significant correlation (P<0.001) to *P. rubescens* or algae were included in the analysis. The significance of added variables was tested by a Monte Carlo permutation test (500 permutations, Pseudo-F=2.008, P<0.0001, alpha=0.05).

References

1. Shimoda, Y. et al. Our current understanding of lake ecosystem response to climate change: What have we really learned from the north temperate deep lakes? *J. Great Lakes Res.* **37**, 173-193 (2011).
2. Foley, B., Jones, I.D., Maberly, S.C. & Rippey, B. Long-term changes in oxygen depletion in a small temperate lake: effects of climate change and eutrophication. *Freshwater Biol.* **57**, 278-289 (2012).
3. Straile, D., Jöhnk, K. & Rossknecht, H. Complex effects of winter warming on the physicochemical characteristics of a deep lake. *Limnol. Oceanogr.* **48**, 1432-1438 (2003).
4. O'Neil, J.M., Davis, T.W., Burford, M.A. & Gobler, C.J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* **14**, 313-334 (2012).
5. Paerl, H.W. & Huisman, J. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **1**, 27-37 (2009).
6. Jöhnk, K.D. et al. Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biol.* **14**, 495-512 (2008).
7. Paerl, H.W., Hall, N.S. & Calandrino, E.S. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci. Tot. Environ*. **409**, 1739-1745 (2011).
8. Ernst, B., Hoeger, S.J., O'Brien, E. & Dietrich, D.R. Abundance and toxicity of *Planktothrix rubescens* in the pre-alpine Lake Ammersee, Germany. *Harmful Algae* **8**, 329-342 (2009).
9. Kurmayer, R., Schober, E., Tonk, L., Visser, P.M. & Christiansen, G. Spatial divergence in the proportions of genes encoding toxic peptide synthesis among populations of the cyanobacterium *Planktothrix* in European lakes. *FEMS Microbiol. Lett.* **317**, 127-137 (2011).

10. Jacquet, S. et al. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural french lake (Lac du Bourget). *Harmful Algae* **4**, 651-672 (2005).
11. Walsby, A.E., Ng, G., Dunn, C. & Davis, A.P. Comparison of the depth where *Planktothrix rubescens* stratifies and the depth where the daily insolation supports its neutral buoyancy. *New Phytol.* **162**, 133-145 (2004).
12. Kurmayer, R. & Jüttner, F. Strategies for the co-existence of zooplankton with the toxic cyanobacterium *Planktothrix rubescen*s in Lake Zürich. *J. Plankt. Res.* **21**, 659-683 (1999).
13. Lewis, W.M., Wurtsbaugh, W.A. & Paerl, H.W. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environ. Sci. Technol.* **45**, 10300-10305 (2011).
14. Rippey, B. & McSorley, C. Oxygen depletion in lake hypolimnia. *Limnol. Oceanogr.* **54**, 905-916 (2009).
15. Wagner, C. & Adrian, R. Cyanobacteria dominance: Quantifying the effects of climate change. *Limnol. Oceanogr.* **54**, 2460-2468 (2009).
16. Winder, M., Reuter, E.R. & Schladow, S.W. Lake warming favours small-sized planktonic diatom species. *Proc. R. Soc. B.* **276**, 427-435 (2009).
17. Bálint, M. et al. Cryptic biodiversity loss linked to global climate change. *Nature Clim. Change* **1**, 313-318 (2011).
18. Dokulil, M.T. & Teubner, K. Cyanobacterial dominance in lakes. *Hydrobiologia* **438**, 1-12 (2000).
19. Walsby, A.E. & Jüttner, F. The uptake of amino acids by the cyanobacterium *Planktothrix rubescens* is stimulated by light at low irradiances. *Microb. Ecol.* **58**, 14-22 (2006).

20. Anneville, O., Gammeter, S. & Straile, D. Phosphorus decrease and climate variability: mediators of synchrony in phytoplankton changes among European peri-alpine lakes. *Freshwat. Biol.* **50**, 1731-1746 (2005).
21. Salcher, M.M., Pernthaler, J., Frater, N. & Posch, T. Vertical and longitudinal distribution patterns of different bacterioplankton populations in a canyon-shaped, deep prealpine lake. *Limnol. Oceanogr.* **56,** 2027-2039 (2011).
22. Campos, A. & Vasconcelos, V. Molecular mechanisms of microcystin toxicity in animal cells. *Int. J. Mol. Sci.* **11**, 268-287 (2010).
23. Hoeger, S.J., Schmid, D., Blom, J.F., Ernst, B. & Dietrich, D.R. Analytical and functional characterization of microcystins [Asp^3]MC-RR and [Asp^3,Dhb^7]MC-RR: Consequences for risk assessment? *Environ. Sci. Technol.* **41**, 2609-2616 (2007).
24. Walsby, A.E. & Schanz, F. Light-dependent growth rate determines changes in the population of *Planktothrix rubescens* over the annual cycle in Lake Zürich, Switzerland. *New Phytol.* **154**, 671-687 (2002).
25. Holland, D.P. & Walsby, A.E. Viability of the cyanobacterium *Planktothrix rubescens* in the cold and dark, related to over-winter survival and summer recruitment in Lake Zurich. *Europ. J. Phycol.* **43**, 179-184 (2008).
26. Zotina, T., Köster, O. & Jüttner, F. Photoheterotrophy and light-dependent uptake of organic and organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance. *Freshwater Biol.* **48**, 1859-1872 (2003).
27. Bright, D.I. & Walsby, A.E. The relationship between critical pressure and width of gas vesicles in isolates of *Planktothrix rubescens* from Lake Zürich. *Microbiol.* **145**, 2769-2775 (1999).
28. Walsby, A.E., Avery, A. & Schanz, F. The critical pressure of gas vesicles in *Planktothrix rubescens* in relation to the depth of winter mixing in Lake Zürich, Switzerland. *J. Plankt. Res.* **20**, 1357-1375 (1998).

29. Bossard, P. et al. Limnological description of the lakes Zürich, Lucerne, and Cadagno. *Aquat. Sci.* **63**, 225-249 (2001).

30. Begert, M., Schlegel, T. & Kirchhofer, W. Homogeneous temperature and precipitation series of Switzerland from 1864 to 2000. *Int. J. Climatol*. **25**, 65-80 (2005).

Correspondence: Correspondence and requests for materials should be addressed to Thomas Posch (posch@limnol.uzh.ch).

Acknowledgements: The long term monitoring was financed by the Zurich Water Supply and the AWEL (Amt für Abfall, Wasser, Energie und Luft). Meteorological data were obtained from the Federal Office of Meteorology and Climatology (MeteoSwiss). The evaluation of the data set was financed by projects of the Swiss National Fund (SNF 31003A_138473; CR22I2_130023/1). We thank M.E. Garneau and R. Psenner for comments on the manuscript.

Contributions: Interpolation of depth profiles and calculations of whole lake contents were done by T. P. The long-term data-set was provided by O. K., as representative of the Zurich Water Supply (WVZ). Statistical analyses were done by M.M.S. All authors discussed the results and created together the design of the figures. T.P and J.P. prepared the manuscript, which was edited by M.M.S and O.K.

Competing financial interests: The authors declare no competing financial interests.

Affiliations

Limnological Station, Institute of Plant Biology, University of Zurich, Seestrasse 187, CH-8802 Kilchberg, Switzerland; Jakob Pernthaler, Thomas Posch & Michaela M. Salcher

Zurich Water Supply (WVZ), Hardhof 9, CH-8021 Zurich, Switzerland; Oliver Köster

Supplementary information

The file contains Supplementary Table S1, and Figures & Legends S1-S6.

Figure legends

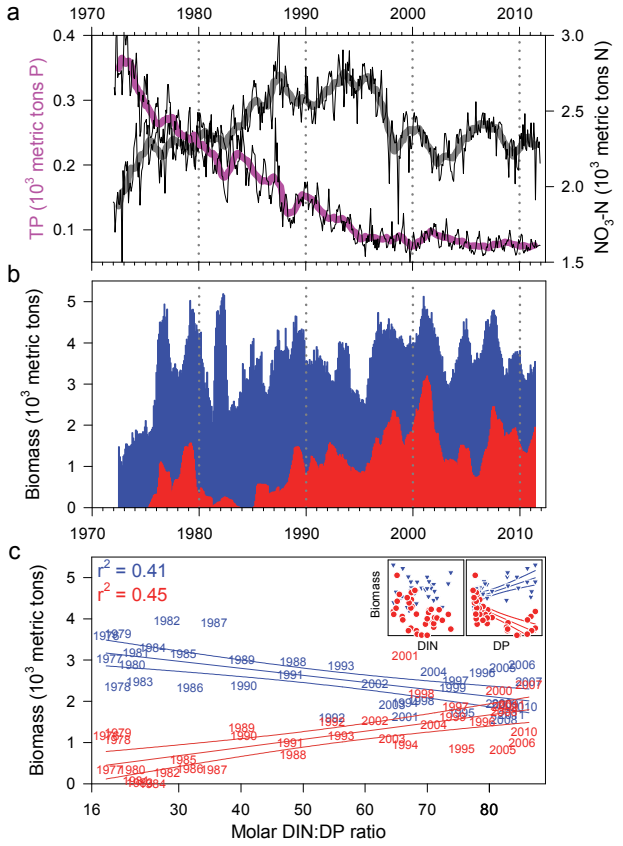
Figure 1 | Trends in whole lake content of nutrients and phytoplankton in Lake Zurich. **a**, Running averages (n=12) for total phosphorus (TP, purple) and for nitrate (NO_3-N, grey) based on monthly values (black). **b**, Running averages (n=12) of whole lake phytoplankton biomass (blue) except for *Planktothrix rubescens* (red). **c**, Relationship between the molar ratio of dissolved nutrients and the annual biomass of organisms (same colours as in **b**) for the years 1976 to 2011. Values are labelled by corresponding years. Only significant ($p<0.0001$) linear regressions are shown. Inserts: relationships between dissolved nutrients (DP = dissolved phosphorus, DIN = dissolved inorganic nitrogen) and organisms.

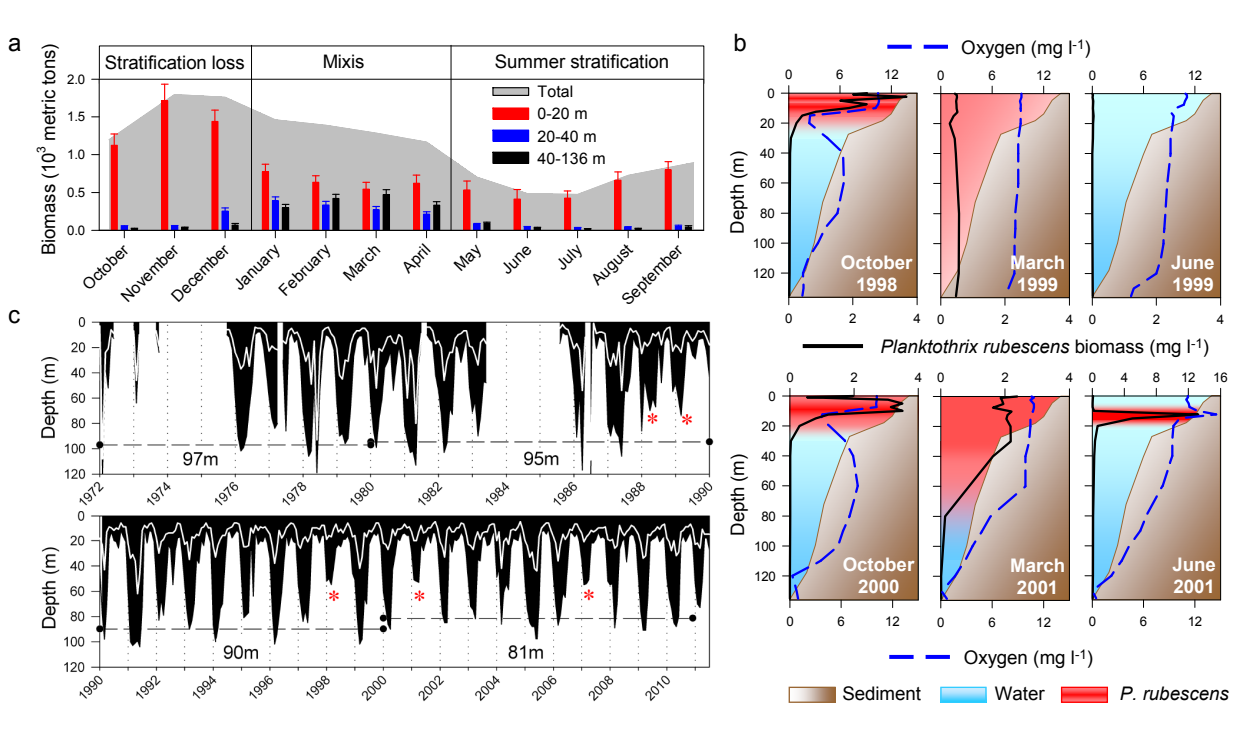
Figure 2 | Seasonality of *Planktothrix rubescens* and its spatial distribution. a, Averages of monthly values during the last 40 years. Total biomass values (grey) are also presented for three spatial zones to highlight depth entrainment of filaments during mixis. **b**, Holomixis in March 1999 as deduced from homogenous oxygen (blue) and *P. rubescens* (black line, schematically: red colouring of water) distribution. After holomixis the cyanobacterium was not detected in June 1999. In contrast, weak mixis in March 2001 was followed by an immense bloom in June 2001 (note different scaling). **c**, Spatial distribution of 90% (black) and 50% (white line) of *P. rubescens* biomass for the period 1972-2011 and decadal depth entrainments during mixis. Asterisks = weakest mixis.

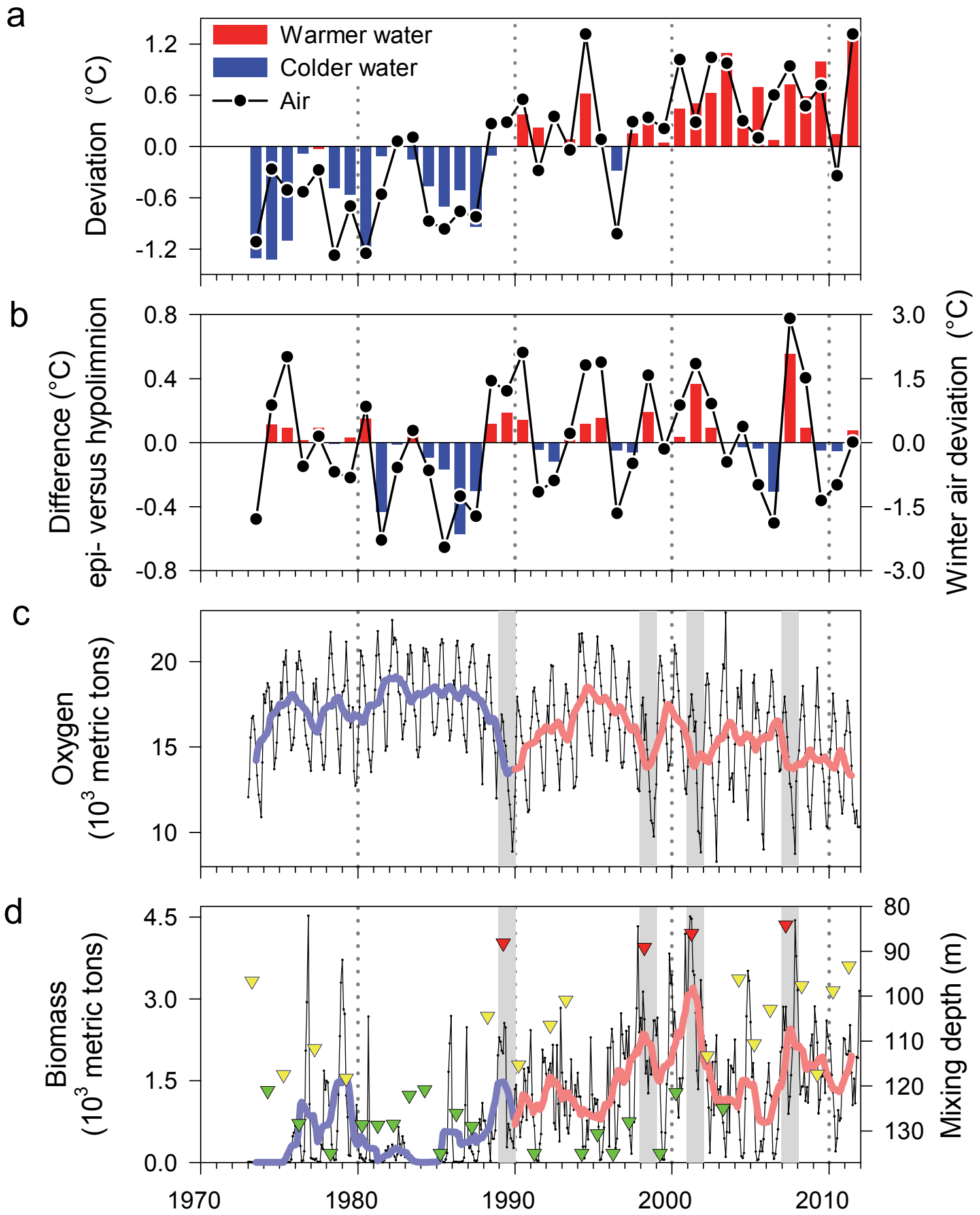
Figure 3 | Context of rising air temperatures with thermal stratification, total lake oxygen content, and biomass of *Planktothrix rubescens*. **a**, Deviations of annual epilimnetic water and air temperatures (black line) from a 40 years average. **b**, Minimal temperature differences (left y-axis) between epi- and hypolimnion during mixis. Blue: colder, red: warmer epi- than hypolimnion. Differences are linked to deviations of winter air temperatures (black line; right y-axis; Supplementary Fig.S2b). **c**, Monthly total oxygen content (black) and running average (blue: colder, red: warmer period). **d**, Monthly total *P. rubescens* biomass (black) and running average (colours as in **c**). Spring mixing depth (triangles; right y-axis)

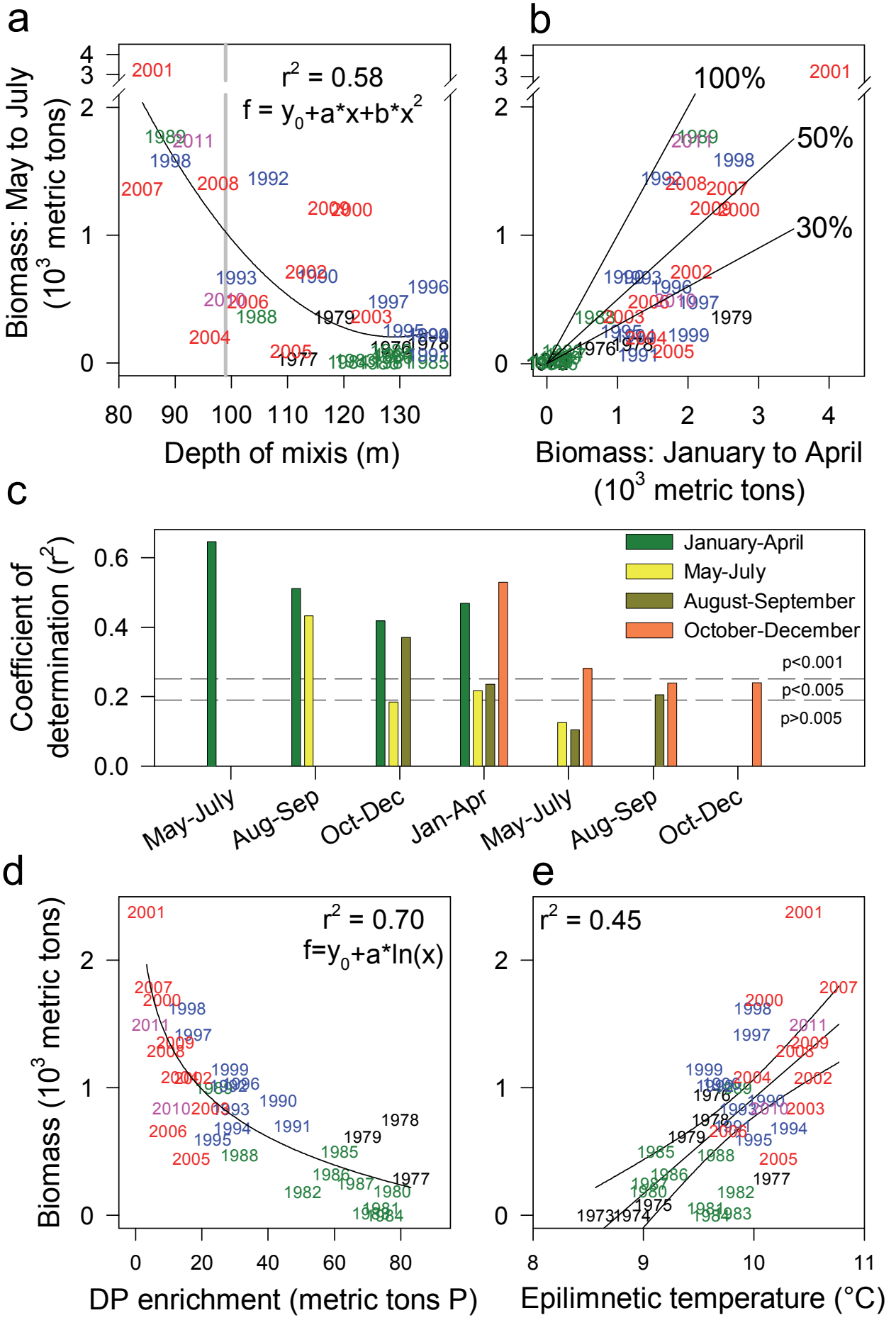
shows years with complete (>120m; green), partial (90-120m, yellow) and weak mixis (<90m, red; grey shaded in **c,d**).

Figure 4 | Relationship between mixing depth, lake warming and growth of *Planktothrix rubescens*. a, Regression between cyanobacterial biomass (summer minimum) and strength of mixis. Grey line: depth below which gas vesicles in cyanobacteria collapse. **b,** Due to partial mixis, up to 100% of the spring biomass can survive. **c**, Influence of seasonal growth phases on each other. Pairwise regression analyses of *P. rubescens* in one growth phase with subsequent phases over 40 years. Significance levels indicated by broken lines. Epilimnetic annual cyanobacterial biomass related to the enrichment with dissolved phosphorus (DP) during mixis (**d**) and to water temperature (**e**). Decadal colour coding: 70s black, 80s green, 90s blue, 2000s red, 2010s purple.


a
1970
1980
1990
2000
2010
0.4
0.3
0.2
0.1
TP (10^3 metric tons P)
3.0
2.5
2.0
1.5
NO3-N (10^3 metric tons N)
b
Biomass (10^3 metric tons)
5
4
3
2
1
0
c
r^2 = 0.41
r^2 = 0.45
Biomass
DIN
DP
Biomass (10^3 metric tons)
16
30
40
50
60
70
80
Molar DIN:DP ratio


a
Stratification loss
Mixis
Summer stratification
Total
0-20 m
20-40 m
40-136 m
Biomass (10^3 metric tons)
October
November
December
January
February
March
April
May
June
July
August
September
b
Oxygen (mg l-1)
Depth (m)
October 1998
March 1999
June 1999
Planktothrix rubescens biomass (mg l-1)
October 2000
March 2001
June 2001
Sediment
Water
P. rubescens
c
Depth (m)
97m
95m
90m
81m


a
Warmer water
Colder water
Air
Deviation (°C)
1.2
0.6
0.0
-0.6
-1.2
b
Difference (°C)
epi- versus hypolimnion
0.8
0.4
0.0
-0.4
-0.8
3.0
1.5
0.0
-1.5
-3.0
Winter air deviation (°C)
c
Oxygen
(10^3 metric tons)
20
15
10
d
Biomass
(10^3 metric tons)
4.5
3.0
1.5
0.0
80
90
100
110
120
130
Mixing depth (m)
1970
1980
1990
2000
2010


a
b
c
d
e
Biomass: May to July (10^3 metric tons)
Depth of mixis (m)
r^2 = 0.58
f = y0+a*x+b*x^2
100%
50%
30%
Biomass: January to April (10^3 metric tons)
Coefficient of determination (r^2)
January-April
May-July
August-September
October-December
p<0.001
p<0.005
p>0.005
May-July
Aug-Sep
Oct-Dec
Jan-Apr
Biomass (10^3 metric tons)
r^2 = 0.70
f=y0+a*ln(x)
DP enrichment (metric tons P)
r^2 = 0.45
Epilimnetic temperature (°C)

Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming

Thomas Posch[1*], Oliver Köster[2], Michaela M. Salcher[1] & Jakob Pernthaler[1]

[1] Limnological Station, Institute of Plant Biology, University of Zurich, Seestrasse 187, CH-8802 Kilchberg, Switzerland.

[2] Zurich Water Supply (WVZ), Hardhof 9, CH-8021 Zurich, Switzerland.

Supplementary Information

Supplementary Table S1, and Figures & Legends S1 – S6

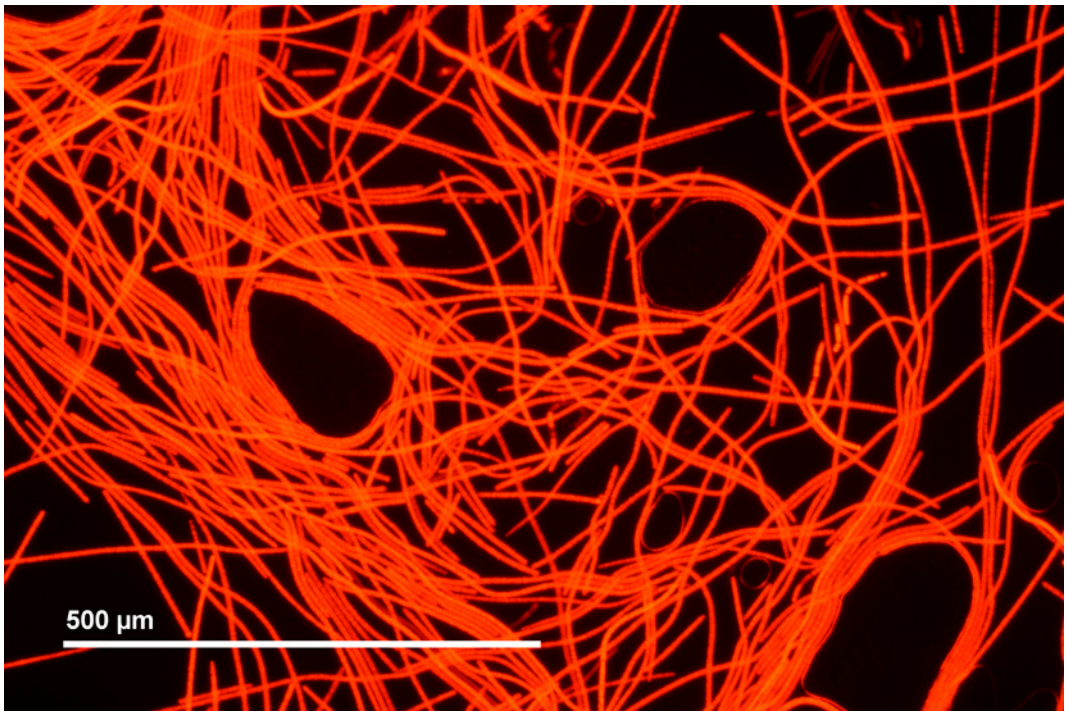
*Corresponding author:
PD Dr. Thomas Posch
Limnological Station, Institute of Plant Biology, University of Zurich
Seestrasse 187, CH-8802 Kilchberg, Switzerland
Phone: 0041 44 716 1224
Fax: 0041 44 716 1225
e-mail: posch@limnol.uzh.ch

Supplementary Table S1 | Statistical analyses of trends in the long term data series.

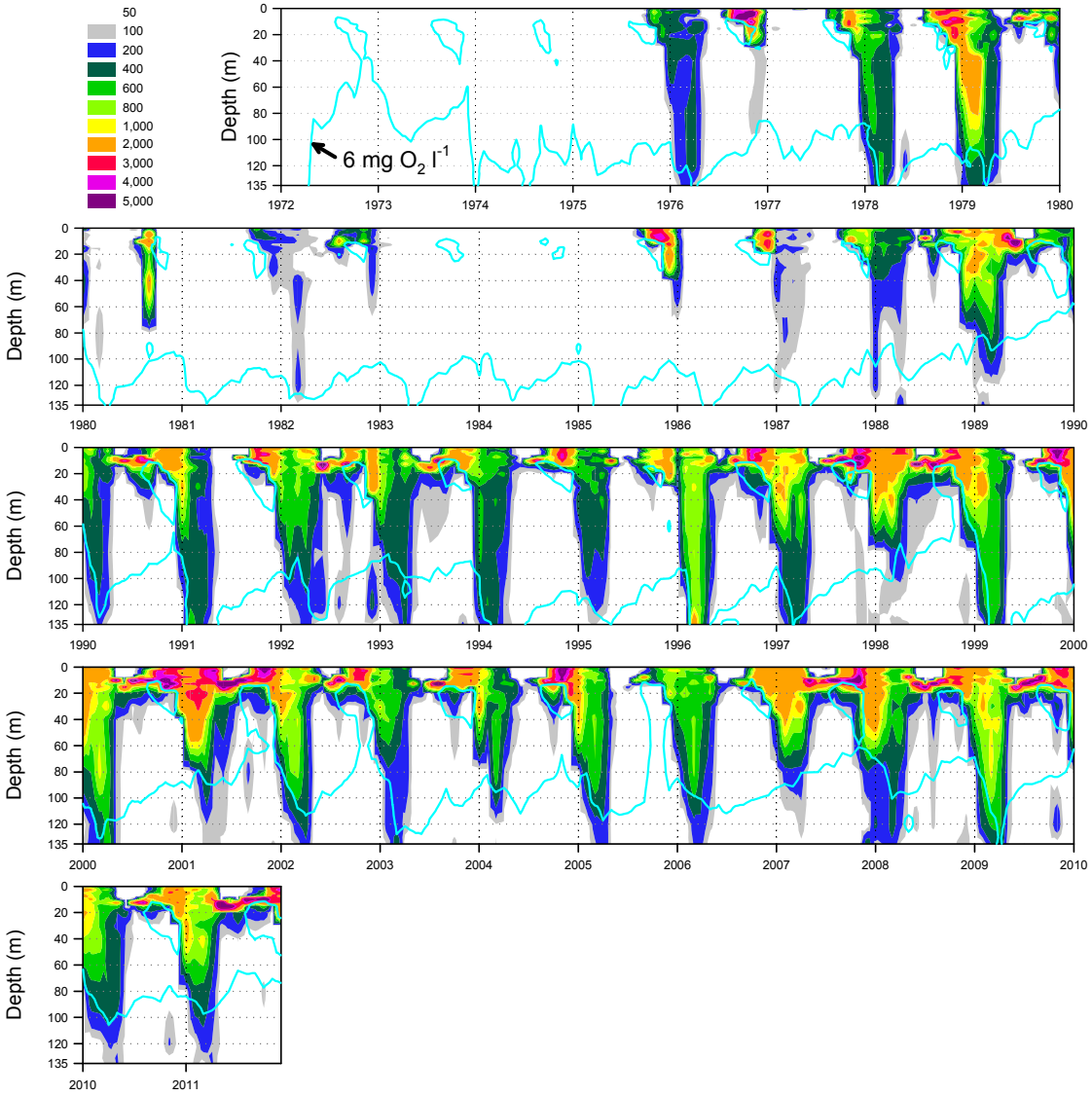
All analyses were performed with the Microsoft EXCEL add-in program XLSTAT 2012 - TIME. Trends in the long term data series were tested by the Mann-Kendall trend test and the seasonal Mann-Kendall test (period = 12). Prior to analysis, data series were tested for autocorrelation. In cases of autocorrelations, Mann-Kendall trend tests were calculated using the Yue and Wang method. P-values of the statistical tests were related to a significance value of alpha = 0.05. The null hypothesis H0 states that there is no trend in the data series, while the alternative hypothesis HA states that there is a trend. In cases where p-values were smaller than alpha=0.05, the null hypothesis HO was rejected.

Parameter	n	Mann Kendall's tau	p-value	Significant trend (Mann-Kendall test)	Autocorrelation Sen's slope	Seasonal Mann-Kendall's tau (period = 12)	p-value	Significant trend (Seasonal Mann-Kendall test)
Biomass								
Total phyto-plankton	432	0.081	0.109	no trend	1.465	0.080	0.087	no trend
Algae without *P. rubescens*	432	-0.138	0.010	negative	-2.046	-0.209	< 0.0001	negative
P. rubescens	432	0.343	< 0.0001	positive	3.749	0.396	< 0.0001	positive
Hypolimnetic oxygen	468	-0.208	< 0.0001	negative	-6.906	-0.341	< 0.0001	negative
Nitrate-N	480	0.066	0.580	no trend	0.165	0.073	0.325	no trend
Ammonium-N	432	-0.056	0.387	no trend	-0.009	-0.065	0.163	no trend
Total phosphorus	479	-0.768	< 0.0001	negative	-0.517	-0.833	< 0.0001	negative
Dissolved phosphorus	432	-0.742	< 0.0001	negative	-0.433	-0.799	< 0.0001	negative
DIN:DP	432	0.714	< 0.0001	positive	0.21	0.762	< 0.0001	positive
Depth of mixis	40	-0.232	0.038	negative	No autocorrelation	n.d.	n.d.	n.d.
90% isocline	36	-0.320	0.007	negative	No autocorrelation	n.d.	n.d.	n.d.

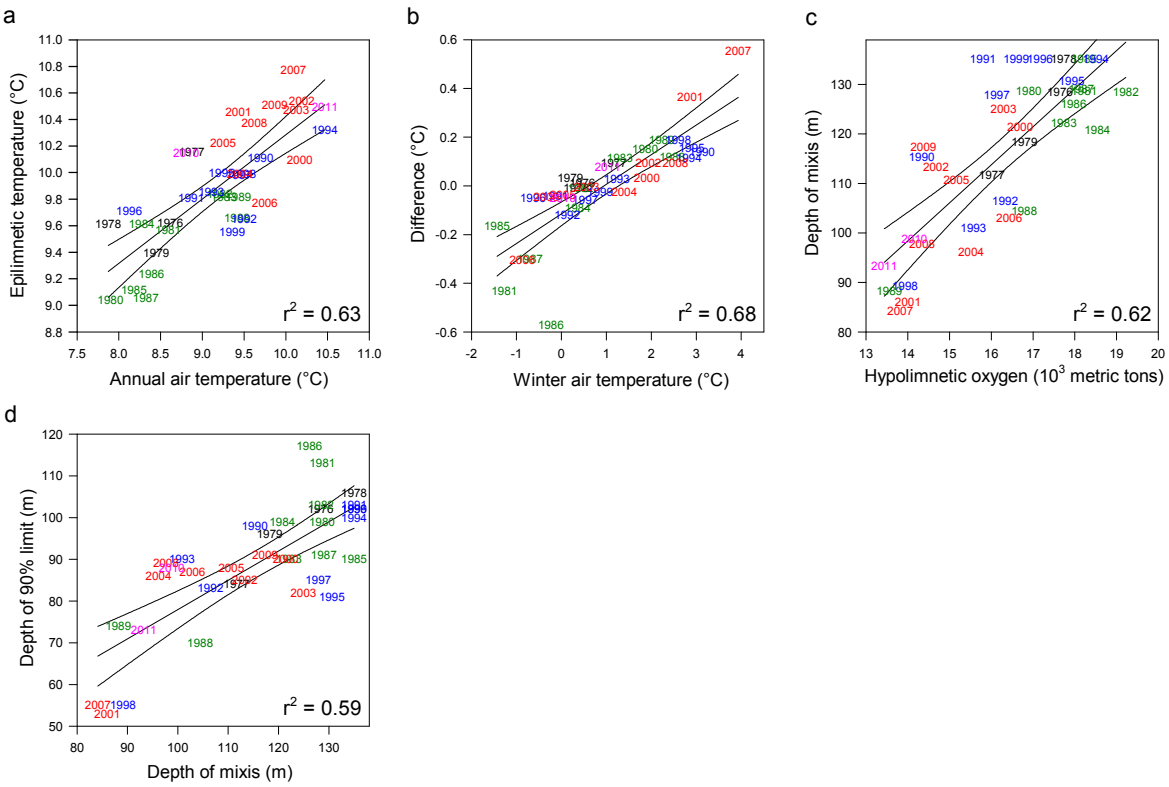
Explanations: 90% isocline = the depth above which 90% of the *P. rubescens* population is located during mixis. Depth of mixis = the depth where a oxygen concentration of 6 mg O_2 l^{-1} was reached during mixis (Supplementary Figs S2, S3 and S4). DIN = dissolved inorganic nitrogen (nitrate-N + ammonium-N). DP = dissolved phosphorus. n.d. = not determined. Phytoplankton = algae and the cyanobacterium *Planktothrix rubescens*.



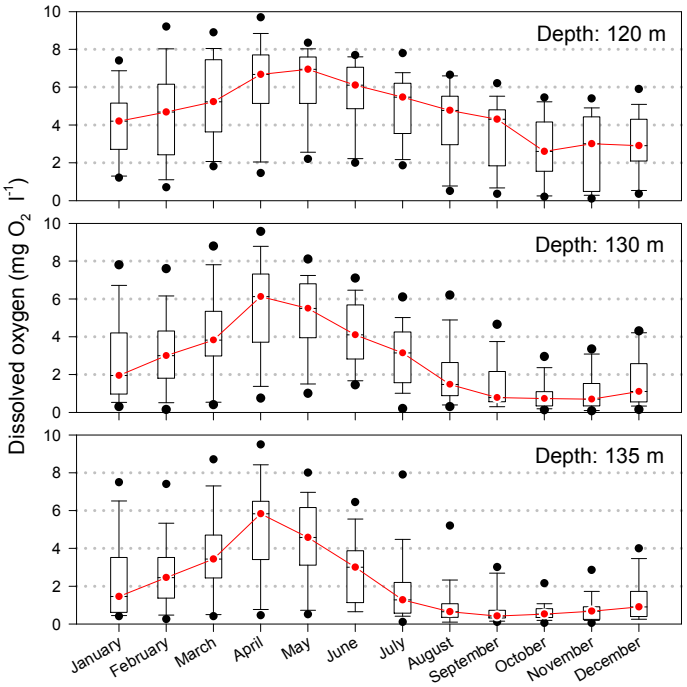
Supplementary Figure S1 | Epifluorescence micrograph of the harmful cyanobacterium *Planktothrix rubescens*. Filaments consist of several hundred single cells (5x5µm), reaching a length up to 3,000µm.



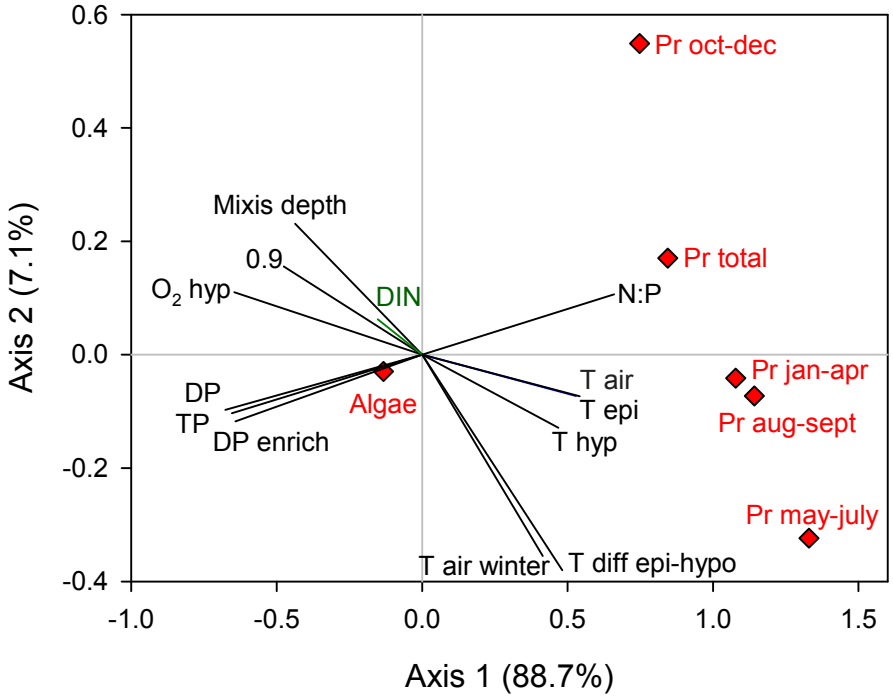
Supplementary Figure S2 | Long-term biomass dynamics of the harmful filamentous cyanobacterium *Planktothrix rubescens* in Lake Zurich (values in µg fresh weight l^{-1}). The figure is based on 14 sampling depths, sampled monthly from 1972 until 2012 (n=6,720). As a proxy for the intensity of water turnover the depth of 6 mg oxygen (O_2) l^{-1} is depicted (cyan line; see also Supplementary Fig.S4). Complete water turnover occurred in years, when the 6 mg O_2 l^{-1} isocline reached the bottom of the lake (136m). Note that each year, a zone with less than 6 mg O_2 l^{-1} also develops between 20-40m. This phenomenon is known as the metalimnetic oxygen minimum, and occurs in many large temperate lakes.



Supplementary Figure S3 | Relationship between lake warming, total oxygen content, mixing depth and entrainment of the harmful cyanobacterium *Planktothrix rubescens* into the hypolimnion (a-d). Linear (**a-d**) correlations between: annual air and annual surface (0-10m) water temperature (**a**), winter air temperature and the minimal temperature difference between epi- and hypolimnion during mixis (**b**), annual hypolimnetic oxygen content and the depth of mixis (6 mg O_2 l^{-1} limit; see also Supplementary Fig.4; **c**), the depth of mixis and the depth above which 90% of the *P. rubescens* population is located during mixis (**d**). The colour coding represents the five decades: 70s black, 80s green, 90s blue, 2000s red, 2010s purple.

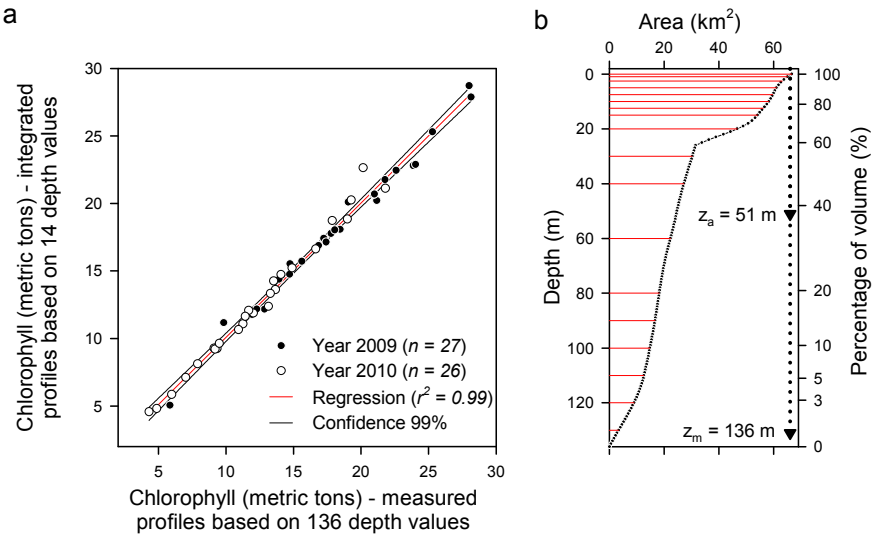


Supplementary Figure S4 | A proxy for mixis intensity: The 6 mg oxygen (O_2) l^{-1} limit. For the period 1972 until 1990 (colder decades), the seasonality of O_2 concentrations is presented for the following sampling depths: 120m, 130m and 135m. Each monthly value evaluates data of 18 years. The bars show the 25th, 50th and 75th percentiles, whiskers stand for the 10th and the 90th percentiles and black points show the outliers. The red line connects the monthly medians. For the 18 years period, the highest O_2 concentrations were reached in April with a median value of 6mg O_2 l^{-1}, even at the deepest point of the lake (135m=one meter above ground).



Supplementary Figure S5 | Redundancy analysis biplot showing the biomasses of algae and the cyanobacterium *Planktothrix rubescens* in relation to the strongest environmental variables (*n*=36 for each parameter). The eigenvalues of the two axes are given in brackets. Abbreviations: 0.9 = the depth above which 90% of the *P. rubescens* population is located, Algae = phytoplankton biomass without *P. rubescens*, DIN = dissolved inorganic nitrogen (NH_4-N + NO_3-N), DP = dissolved phosphorus, DP enrich = enrichment of the epilimnion with DP due to mixis, Mixis depth = the depth down to which oxygen concentrations of 6 mg l^{-1} were reached after mixis, N:P = molar ratio of dissolved nutrients (DIN:DP), O_2 hyp = hypolimnetic oxygen, Pr = total annual biomass of *P. rubescens* (the seasonal growth phases are also presented), T air = air temperature, T air winter = winter air temperature (December to February), T diff epi-hypo = minimal water temperature difference between epi- and hypolimnion during mixis, T epi = epilimnetic water temperature, T hyp = hypolimnetic water temperature, TP = total phosphorus.

Prior to statistical analyses, data were log(x+2)-transformed to obtain normal distribution. Redundancy analysis was used to determine the effects of environmental variables on algal and *P. rubescens* densities. Only those variables exhibiting a significant correlation (P<0.001) to *P. rubescens* or algae were included in the analysis. The significance of added variables was tested by a Monte Carlo permutation test (500 permutations, Pseudo-F=2.008, P<0.0001, alpha=0.05). Analyses were performed with the Microsoft EXCEL add-in program XLSTAT-ADA.



Supplementary Figure S6 | **a**, Significance test (linear regression) of the whole lake content estimation based on the 14 standard sampling depths (0, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 80, 120, 135m) for the analysis of biological parameters. In two years (2009 and 2010), biweekly continuous profiles (0-136m) of chlorophyll *a* (Chl *a*) concentrations were determined with a continuously recording submersible spectro-fluorometer (TS-16-12, bbe Moldaenke GmbH). The total Chl *a* content was calculated based on 136 measured depth values (x-axis) and compared to the calculation based on the pre-defined 14 depth values (y-axis). Both calculations are highly correlated (r^2=0.99). **b**, Bathymetric information of Lake Zurich (lake areas from 0-136m and volume distribution along the vertical profile). Horizontal red lines indicate the 19 depths (0, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 60, 80, 90, 100, 110, 120, 130, 135m) which have been sampled monthly for physical-chemical analyses during the last 40 years. Abbreviations: z_a = average depth, z_m = maximal depth.